#8 3RD

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application For Renewal of Research Grant

(Use extra pages as needed)

First Renewal ☐ Second Renewal ☒ Date: July 15, 1973

1. Principal Investigator (give title and degrees):

Edwin R. Fisher, M.D.,Director of Laboratories, Shadyside Hospital, 5230 Centre Avenue, Pittsburgh, Pa.; Professor of Pathology, University of Pittsburgh, Pittsburgh, Pa.

2. Institution & address:

Shadyside Hospital
5230 Centre Avenue
Pittsburgh, Pa. 15232

3. Department(s) where research will be done or collaboration provided:

Research will be done in Dept. of Pathology, Shadyside Hospital.
Collaborative help provided by Mark Wholey, M.D., Director, Division of Radiology, Shadyside Hospital.

4. Short title of study:

Effect of Tobacco Smoke and Nicotine on Structure and Function of Coronary Arteries and Plasma Lipids in Rabbits.

5. Proposed renewal date:

Anniversary Date - October 1, 1973

6. How results to date have changed earlier specific research aims:

None

7. How results to date have changed earlier working hypothesis:

None

8. Any additional facilities now required? Describe briefly:

None

9. Any changes in personnel? Append biographical sketches of new key professional personnel:

See Page 3, 13A, Technical

10. Append outline of experimental protocol for ensuing year.
11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

Influence of Nicotine on Experimental Atherosclerosis and Its Determinants, by Edwin R. Fisher, M.D., R. Rothstein, M.S., Mark H. Wholey, M.D., and R. Nelson, M.S. Archives of Pathology. In press.

12. Summary progress report (append in standard form, as separate document, unless recently submitted)

13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
Edwin R. Fisher, M.D.	35	----
Mark Wholey, M.D.	10	----
Technical		
Marie Tomko (Histotechnician)	85	7620
Virginia Malek (EM Technician)	85	6000
Dolores Van Holt (Histotechnician)	25	2000
Yang ksien Ke, Ph.D. (Chief, Experimental Path.)	25	2000
Sub-Total for A		17620

B. Consumable supplies (by major categories)	
Animals	1000
Histopath supplies	500
Electron microscopy supplies	750
Radiologic supplies	750
Drugs (Cholesterol)	500
Sub-Total for B	3500

C. Other expenses (itemize)	
Publication	200
Reference Cigarettes	400
Sub-Total for C	600
Running Total of A + B + C	21,720

D. Permanent equipment (itemize)

None

Sub-Total for D	----
E. Indirect costs (15% of A+B+C)	3258
Total direct cost	24,978

14. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Ultrastructural Studies in Human and Experimental Pathology	Shadyside Hospital Laboratory Research Fund	10,000/ annum	yearly

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

100354185 6

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Edwin R. Fisher, M.D.

Signature [signature] Date 7/19/73

Telephone 412 (Area Code) 622 (Number) 2315 (Extension)

Checks payable to

David Haldeman
Director of Fiscal Affairs

Mailing address for check:

Shadyside Hospital
5230 Centre Avenue
Pittsburgh, Pa. 15232

Responsible officer of institution

Typed Name David Haldeman

Title Director of Fiscal Affairs

Signature [signature] Date 7/19/73

Telephone 412 (Area Code) 622 (Number) 2036 (Extension)

CTR GRANT #839

Progress Report No. 2

Edwin R. Fisher, M.D.
Director of Laboratories
Shadyside Hospital
5230 Centre Avenue
Pittsburgh, Pennsylvania 15232

EFFECT OF TOBACCO SMOKE AND NICOTINE ON STRUCTURE AND FUNCTION OF CORONARY ARTERIES AND PLASMA LIPIDS IN RABBITS

Since submission of the last Progress Report, the protocol concerned with the effect of nicotine administration on the structure and function of coronary arteries and plasma lipids in rabbits with and without various discriminants of atherosclerosis has been completed. A manuscript describing this investigation and the results obtained has already been accepted for publication in the Archives of Pathology. A copy of the pre-print is enclosed for perusal.

During the past year a "cigarette smoking machine" applicable for use in rabbits has been obtained to perform the protocol as originally outlined in regard to this form of nicotine consumption. Such studies are now in progress including smoking rabbits with and without induced renal hypertension and/or cholesterol atherosclerosis. Progress in this regard is relatively slow since the machine utilized accomodates only two animals per each exposure and each animal in all groups consumes 1 cigarette per day. Nevertheless, thus far the findings which are preliminary in this regard appear to parallel those observed following nicotine administration.

In addition, we have considered it worthwhile to obtain data on animals subjected to cigarette smoking for longer periods than originally outlined. Not only will this extended period of observation be more meaningful insofar as the cardiovascular effects of this form of nicotine administration but it will also allow us to obtain some meaningful histologic and ultrastructural

(Continued)

information concerning the lungs in such animals. Therefore, in addition to the relatively short term observations of 2-3 months as indicated in the original protocol, some animals will be subjected to the effects of smoking for 9-12 months. Indeed, some animals have already been sacrificed after 10 months of cigarette smoking. Although the number in this category at present are relatively few, nevertheless preliminary study has failed to disclose significant cardiovascular or pulmonary alterations related to such treatment.

Aside from the extended period of observation and examination of the lungs in animals subjected to cigarette smoking, it is our intention to adhere to the protocol as originally submitted.

INFLUENCE OF NICOTINE ON EXPERIMENTAL ATHEROSCLEROSIS AND ITS DETERMINANTS*

Edwin R. Fisher, M.D., R. Rothstein, M.S., Mark H.Wholey, M.D. and R. Nelson, M.S.

*Supported by Grant #839R1 from the Council for Tobbacco Research-U.S.A.

From the Departments of Pathology and Radiology Shadyside Hospital and University of Pittsburgh, Pittsburgh, Pennsylvania.

Address for reprints: Edwin R. Fisher, M. D., Institute of Pathology, Shadyside Hospital, 5230 Centre Avenue, Pittsburgh, Pennsylvania .

ABSTRACT

A realistic daily pharmacologic dose of nicotine failed to quantitatively or qualitatively affect the atherosclerosis of aorta and extramural as well as intramural coronary arteries, visceral lesions, or serum lipids in normotensive or hypertensive rabbits with and without a dietary cholesterol supplement. No difference in the appearance of coronary angiograms could be appreciated in nicotine-treated rabbits with and without atherosclerosis. This technic did reveal less tortuous coronary arteries in all hypertensive rabbits which was reflected histologically by slightly greater luminal areas than in normotensive animals. Hypertension augmented the atherosclerotic process in the aorta and coronary arteries of cholesterol-fed rabbits. Nicotine failed to influence the induction or maintenance of renal hypertension. Although the clinical significance of these findings is uncertain, nevertheless they provoke the need for further inquiry concerning the role of nicotine, vis a vis cigarette smoking, and other determinants in the development of atherosclerotic heart disease in man.

There have been many epidemiological studies which reveal a significant association between cigarette smoking (CS) and the morbidity and mortality from arteriosclerotic heart disease (AsHD). It is noteworthy that this relationship is less conspicuous than that for CS and diseases of the respiratory system. Further a causal role of CS to AsHD is less clear. Most antagonists to views relating CS to AsHD propose a common genetic factor which may be responsible for both. Support for this view appears from the outstanding "twin studies" of Lundman.[1] Some skepticism concerning the role of CS in AsHD has also been derived from the inconsistencies and somewhat paradoxical results obtained from epidemiological studies considering the duration of CS and events occuring in ex-smokers when compared to non-smoking populations.[2,3,4]

Results of pharmacologic investigations concerning the effect of CS or nicotine on the cardiovascular system often reveal divergent results which, at least in part, appear to be related to differences in dosage of nicotine utilized and experimental technics employed. Also, most of these studies might be regarded as acute and therefore unrevealing with respect to such a chronic disorder as AsHD. Nevertheless, there is evidence which indicates that the cardiovascular effects of CS are synonymous with that of nicotine.[5,6,7] In man short term studies have disclosed a slight pressor effect, tachycardia and an increase in cardiac output following inhalation of cigarette smoke or intravenous injections of nicotine.[8,9,10] Although some[11] have found a more prolonged pressor effect and tachycardia in chronic smokers than in non-smokers following CS others have denied such differences.[12,13] Studies of CS or nicotine in animals reveal comparable cardiovascular effects to those

There have been many epidemiological studies which reveal a similarity observed in man and are attributed to the stimulating effect of nicotine on the sympathetic nervous system and to catecholamine release.[14,15,16] The net effect of these actions has been interpreted to represent an adverse increased oxygen demand by the heart. It is noteworthy that doses of nicotine in dogs which are apparently devoid of systemic effects not only reproduce these changes but also result in increased coronary blood flow.[17] This latter phenomenon appears to be confirmed by most recent studies concerning the effects of nicotine and/or CS on the cardiovascular system.[18-22]

Retrospective pathological studies in man have for the most part disclosed varying degrees of increased aortic and coronary atherosclerosis in heavy smokers (generally more than 20 cigarettes per day) than in non-smokers.[23-26] The age of men exhibiting sudden death due to a first episode of AsHD has been found to be 16 years less in heavy smokers than non-smokers and intermediate for ex-smokers and light smokers.[27] However, it should be indicated that most of these studies failed to consider other determinants such as serum lipids and hypertension which may influence the development of AsHD.

There have been surprisingly few experimental studies concerning the effect of CS or nicotine on the development of cardiovascular disease.[28-35] The results have been conflicting and analysis of their significance is hampered by differences in species and technics employed as well as varying doses of nicotine administered. Generally, the experimental designs have failed to consider other parameters which might influence or play a role in atherogenesis.

The purported significance of serum lipids in the pathogenesis of AsHD needs no elaboration. The effect of CS on this parameter has received a relatively modest amount of attention. Again, the results of investigations in this regard are inconsistent. Some have failed to note any immediate effects of CS in man upon serum cholesterol, phospholipids or triglycerides.[36,37] Free fatty acids apparently increase after smoking although Frankl et al [38] believe this may represent an anxiety reaction to the tests being performed. Although cholesterol may be unaltered after smoking it is claimed by some,[39-41] but not others, [42] that habitual smokers exhibit higher levels of cholesterol and beta lipoproteins than non-smokers. In animals, administration of nicotine has been noted to result in an immediate rise in serum triglycerides but not cholesterol, whereas, the converse appears to obtain in more chronic experiments.[37] A decreased rate of cholesterol synthesis as well as decrease of hepatic and myocardial cholesterol content has been observed in nicotine-treated dogs. [43] A few studies have been performed concerning the effect of CS on coagulation since alteration of this system may also represent another of the many factors concerned with atherogenesis. Generally, there is little or no effect on blood coagulation in smokers or after smoking [44] although increased platelet stickiness [45] and in vitro thrombus formation [45,46] have been recorded.

The purpose of this present study was to investigate the pathologic effects of nicotine on cardiovascular and other tissues in rabbits as revealed by coronary angiography and appropriate histologic, histochemical and ultrastructural technics. Such studies as well as those of serum lipids were performed in untreated rabbits and those subjected to such

determinants of AsHD as hypercholesterolemia and/or hypertension.

MATERIALS AND METHODS

Eighty-seven adult male and female albino rabbits weighing 2-2.5 kg. survived or satisified the requirements of the experiment. These comprised the following groups. Group I consisted of 16 that received Purina rabbit chow containing 2% cholesterol. Group II consisted of 10 that received the regular ration without added cholesterol but were given twice daily subcutaneous injections of 0.5 mg of nicotine dissolved in physiologic saline. Preliminary studies revealed that of 0.5 mg. of nicotine caused a transient rise of 15-20 mm. Hg. in blood pressure and tachycardia in normal rabbits. This total daily dose is estimated on a weight basis to be equivalent to smoking approximately 35 cigarettes per day in man considering that 1 mg. of nicotine is absorbed from 1 inhaled cigarette.[47] There were 12 in Group III in which hypertension was successfuly induced by the method of Page[48] except that both unilateral nephrectomy and cellophage enclosure of the contralateral kidney were performed in one stage. Group IV consisted of 12 hypertensive rabbits that received nicotine as described above. Group V was comprised of 10 cholesterol-fed hypertensive animals and Group VI, 15 similarly fed normotensive rabbits that received nicotine as above. Group VII consisted of 12 hypertensive cholesterol-fed animals that also received nicotine.

Animals were sacrificed 90 days following operation and/or the administration of the cholesterol diet or nicotine injections.

Blood pressure was estimated indirectly at biweekly intervals by the ear capsule technic of Grant and Rothschild.[49]

All biochemical reactions were performed on aortic blood obtained at the time of sacrifice after an overnight fast. Total lipids were determined by the phospho-vanillin reaction; triglycerides by the automated colorimetric periodate reaction; total cholesterol by the method of Lieberman and Burchard and phospholipids by differentiation. Beta and alpha lipoproteins were calculated as percent of lipoproteins from cellulose acetate electrophoretograms stained with oil red O and total proteins by the biuret reaction. Serum calcium, phosphorus, urea N, bilirubin, alkaline phosphatase, LDH, SGOT and electrolytes by the methods utilized with the "Autoanalyzer".

Coronary angiography was performed by catherization of the left femoral artery. The catheter was positioned either selectively in the left coronary orifice or at the root of the aorta at the level of the aortic cusps by television fluoroscopy. Injections of methyl glucamine diatrizoate (Renografin 76) was accomplished by flow rate control at 6 ml/sec for a total of 8 ml. In instances of selective angiography 1 ml was delivered by manual controlled flow. Films were exposed on a Franklin roll film changer at a rate of 4/sec for two seconds. At least 5 animals in each group had successful coronary angiograms performed just prior to sacrifice.

At the time of sacrifice the heart, liver, adrenals and spleen were cleaned and weighed. The degree of aortic atherosclerosis was determined arbitratily by computing the average grades of atherosclerosis of both the thoracic and abdominal portions as described previously.[50]

Blocks of heart, lungs, aorta, small intestine, pancreas, spleen, kidneys, gonads, and thyroid were fixed in 10% neutral formalin; those of adrenal in both formalin and Orth's fluid and those of the extramural branches of the coronary arteries in gluteraldehyde. Paraffin sections were prepared in the usual manner and stained with hematoxylin and eosin. In addition, sections of coronary arteries, heart, and aorta were stained with thionin pH 4, 1:10,000 for estimation of metachromasia and orcein elastica and von Kossa calcium methods. Adrenals were stained by the ferric-ferricyanide chromaffin technic. Portions of coronary arteries fixed in gluteraldehyde were post fixed in 1% osmium tetroxide, dehydrated and imbedded in Maraglas. Ultrathin sections were examined by an EM 200 electron microscope.

The luminal area of extramural branches was computed from similarly magnified photographs of these structures by the formula A= ab. Comparisons of such measurements between groups were expressed as ratios.

Significance of differences between groups was estimated by the Student"t" test.

RESULTS

All animals exhibited a gain in body weight during the experimental period (Table I). This was least pronounced in hypertensive members. Nicotine had no effect on body weight.

Blood pressure was comparably ($P=>.05$) but significantly ($P=<.01$) elevated in hypertensive animals of all groups. Nicotine and/or hypercholesterolemia failed to affect the level of hypertension (Table I).

Total serum lipids, total cholesterol, triglycerides, phospholipids, and beta lipoproteins were significantly ($P=<.01$) but comparably ($P=>.05$) elevated in animals of all groups receiving the cholesterol diet. The administration of nicotine and/or presence of hypertension had no effect on these serum lipids in non-cholesterol-fed animal ($P=>.05$). Total serum proteins appeared unaltered and similar in all groups (Table II).

No changes in serum calcium, phosphorus, bilirubin, or alkaline phosphatase were evident. LDH and SGOT although greater than that observed in man was in the normal range (LDH 175-350; SGOT 75-175) for control rabbits in all groups studied. Serum electrolytes were comparable in all groups. Urea N was slightly but not significantly elevated only in rabbits of Group III that were subjected to the induction of renal hypertension.

Weight of the heart was significantly ($P=<.01$) increased in those groups of animals with hypertension, that of the adrenals only in those groups receiving the cholesterol diet ($P=<.01$) (Table I).

Coronary angiography disclosed foci of atherosclerotic beading, and narrowing of one or more coronary arteries only in cholesterol-fed rabbits (Figs 1A & B, 2A & B). Such changes occured at varying sites along the affected artery and were most frequent in the circumflex branch of the left coronary which appeared to be the predominant vessel in the rabbit. The

degree of change appeared to be unaffected by the administration of nicotine, but was more pronounced in the hypertensive, cholesterol-fed animals. Angiographically, coronaries of otherwise untreated hypertensive rabbits were less tortuous than those of other groups.

Hypertensive, cholesterol-fed rabbits exhibited more extensive aortic atherosclerosis than normotensive cholesterol-fed animals (Fig 3). Nicotine administration failed to influence the severity of aortic atherosclerosis. No atherosclerosis or other vascular changes were observed in nicotine-treated or hypertensive animals not receiving the cholesterol diet.

The histopathological appearances, degree of elastica alteration and intimal calcium deposition of the lesions of the aorta, coronary and other arteries were qualitatively similar in all cholesterol-fed rabbits regardless of presence or absence of hypertension or administration of nicotine and have been recounted in detail previously.[50] Metachromasia appeared increased in aortas from all hypertensive rabbits whether or not they received the cholesterol diet. In these instances the metachromatic material was evident throughout the entire medial coat as well as in the intimal lesions of cholesterol-fed members. A slight increase in metachromasia was apparent in the media of the extramural branches of the coronary arteries of all hypertensive animals only, but this change was less consistent in other systemic arteries of these animals. No effect of nicotine treatment on the degree of metachromasia was appreciated.

A qualitative difference in the type of intimal atherosclerosis existed between the lesions of extramural coronary and distributing arteries of

similar size (600-1200 u luminal diameter) (Fig 4) and the intramural branches of the coronary arteries with luminal diameter of 40-660 u. (Fig 5). In the former, the atheromatous lesions resembled those of the aorta with distinct foam cells, varying amounts of collagen and amorphous lipid deposits, whereas those of intramural branches consisted almost exclusively of large, irregularly shaped acellular collections of optically clear lipid with indistinct cells borders and only occasional nuclei. This lesion often appeared to obliterate the lumen. The media of these involved vessels was markedly thinned. The ratio of the luminal area of extramural coronary arteries of hypertensive rabbits to that of normotensive members was 1.5-1.7 whereas that of other groups more closely approximated 1.

The ultrastructural features of cholesterol atheroma in coronary arteries were comparable to those described previously in aortas of cholesterol-fed rabbits by others. [51-53] Nicotine and/or hypertension failed to influence these changes in cholesterol-fed animals or the normal appearance of these vessels in those receiving the non-cholesterol diet.

Sections of heart from approximately $\frac{1}{4}$ of the rabbits from cholesterol-fed groups exhibited miliary infarcts (Figs 6 & 7) or foci of subendocardial necrosis in the myocardium of the left ventricle. In addition, interstitial infiltrates of foam cells with or without other inflammatory infiltrate were also observed in $\frac{1}{4}$ of cholesterol-fed animals. These appeared to be most pronounced in rabbits with hypertension and not related to nicotine treatment or levels of serum lipids.

No distinct qualitative or quantitative differences in lipid deposits in other viscera were apparent in any group studied or in the numbers of arteries in them involved with atheromatous plaques in cholesterol-fed rabbits. No differences in ferric-ferricyanide reactive chromaffin tissue of adrenal medullas was apparent in any group studied.

Although the numbers of each sex were small in each group there did not appear to be any qualitative or quantitative differences in the atherosclerosis, angiographic, or biochemical findings in males and females.

No increase in incidence of spontaneous medial lesions was encountered in any group.

DISCUSSION

The results of these studies fail to disclose any significant influence of nicotine on the severity, histopathologic, untrastructural, histochemical or angiographic features of aortas and coronary arteries or serum lipids of otherwise untreated rabbits or those subjected to such determinants of atherosclerosis as hypercholesterolemia and/or hypertension. Similarly, the incidence and/or severity of rare foci of myocardial necrosis was unaffected by nicotine administration. These myocardial changes have not been observed with any degree of frequency in cholesterol-fed rabbits not receiving other thrombogenic factors, but was relatively conspicuous in this study. On the other hand, overt myocardial infarction was not observed and might be explained by the predominant and often exclusive involvement of the circumflex branch of the coronary system, a situation unlike that observed in man.

The failure of nicotine to augment the atherosclerotic process even in cholesterol-fed rabbits with hypertension is in agreement with results of previous studies in the rat [32] and the dog [37] which fail to ascribe any pathologic changes in the cardiovascular system to nicotine. It should be noted that these species are quite resistant to atherosclerosis even in the presence of hypercholesterolemia.

In the rabbit, Wenzel et al [34,35] using graded doses of nicotine in drinking water failed to discern any effect of this agent on aortic atherosclerosis. However, they did record "thickening and fibrosis in small branches of coronaries" following nicotine treatment although details in this regard were not presented or depicted. Further, occlusive coronary changes were encountered in nicotine-treated, cholesterol-fed rabbits accompanied by myocardial necrosis. Since this was not apparent in nicotine treated members not fed cholesterol they proposed that these changes were due to some interaction between the two. Not only do these authors disregard the possibility that these changes may be due to cholesterolemia per se, as is shown in this study, but also it is evident that the references to atherosclerotic involvement is concerned with intramural branches of the coronary arteries only. In addition, these investigators noted that nicotine produced a rise in cholesterol in male but not in female rabbits [33] whereas in our study no sex differences were observed, albeit the numbers of each were few. Stefanovich et al. [28] found slightly greater aortic atherosclerosis and serum cholesterol in nicotine, cholesterol-fed rabbits. It does appear significant that these investigators utilized

a dose of nicotine which by our estimates appear equivalent to approximately 175 cigarettes a day in man or 5 fold that used in these present studies. It is of interest that they also observed an increase in serum phospholipids, which in our experience with cholesterol atherosclerosis in rabbits is attendant with a decreased severity of the vascular process.[54,55] The failure of nicotine to influence aortic acid mucopolysaccharide content is in accord with our histochemical findings in the nicotine-treated animals. Increases in this moiety have been noted in this and other studies by us in situations in which the atherosclerotic process in rabbits is augmented.[50,55,56] The studies of Lellouch et al[30] are difficult to evaluate since these investigators, utilizing a dose of nicotine equivalent to 525 cigarettes per day, found this agent to induce aortic subendothelial fibrosis which was unrelated to cholesterol-feeding, but mimicked that produced by adrenalin and was inhibited by monamine oxidase inhibitors. This lesion is unique for we have been unable to find any previous or subsequent accounts of a similar aortic change. Hass and associates[31] similarly utilized an exceedingly high dose of nicotine as well as vitamin D in cholesterol-fed rabbits. They observed a pronounced medial effect on the aorta and other peripheral arteries including the coronaries as well as intimal change including thromboses in these latter vessels. It is quite apparent that one of the major sources of divergence of the results of these studies from our findings may reside largely in experimental design. particularly that concerned with the dose of the nicotine utilized which often appears to be in excess of that which may be regarded as realistic.

It is of interest that nicotine failed to affect the induction or maintenance of renal hypertension in the rabbit. Wenzel and Azmeh [57] noted similar results on the induction of renal hypertension in rats treated with nicotine, but a subsequent depressor effect after long-term treatment. Again the dose of nicotine was much greater than that utilized in our studies. It is well recognized that low doses of nicotine may be stimulating whereas the converse obtains with higher doses.

This study reaffirms the aggravating effect of hypertension on cholesterol-atherosclerosis. The coronary as well as other peripheral arteries in hypertensive rabbits not receiving the cholesterol diet and therefore lacking atherosclerosis, appeared less tortuous by angiography. This was reflected histologically by their slightly greater luminal area suggesting that uncomplicated hypertension may actually increase coronary blood flow.

It is appreciated that the results in the present study which fail to reveal any adverse effect of nicotine on the structural integrity of the cardiovascular system in rabbits with or without some other determinants of AsHD may not be applicable to the situation in man or other species. Nevertheless, they provoke the need for further study and scrutiny regarding the purported causal role of CS or nicotine in AsHD.

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TABLE I. BODY WEIGHT, BLOOD PRESSURE AND ORGAN WEIGHTS OF CHOLESTEROL-FED, HYPERTENSIVE, NICOTINE-TREATED RABBITS

Group	Change body wt. (Kg)	Blood pressure mmHg		Organ weights (Gm)		
		Init.	Final	Heart	Liver	Adrenals
I. Cholesterol-fed	+1.0	96±12	104±10	5.6±.8	95±30	1.070 ± .240
II. Nicotine	+1.2	105±8	108±11	6.5± .9	104±20	.490±.170
III. Hypertensive	+.7	105±8	145±12	8.8±.4	99±30	.490±.240
IV. Hypert.+Nicotine	+.8	103±7	140±7	8.6±.8	90±30	.500±.130
V. Hypert. + Cholesterol	+.8	100±10	148±14	8.5±.6	100±32	.988±.120
VI. Cholesterol+Nicotine	+.9	110±7	110±11	6.0±.7	105±25	.990±.290
VII. Hypert.+Chol.+Nico.	+.7	105±10	138±8	8.8±.7	108±28	1.200±.320

TABLE II. SERUM LIPIDS, TOTAL PROTEIN IN (TP)/CHOLESTEROL-FED, HYPERTENSIVE AND NICOTINE-TREATED RABBITS

Group	Tot.Lipids (mg%)	Triglyc. (mg%)	Cholesterol (mg%)	P.Lipids (mg%)	Beta Lipoprot. (%)	Alpha Lipoprot. (%)	T.P. (Gm)
I.Cholesterol-fed	2402±500	386±102	1421±469	595±105	88	12	6.5±.6
II. Nicotine	314±74	95±209	65±20	154±80	45	55	6.2±.7
III. Hypertensive	320±63	112±35	57±76	151±76	55	45	6.6±.7
IV. Hypert.+Nicotine	320±65	78±20	110±40	132±64	50	50	6.5±.3
V.Hypert.+Cholesterol	2308±340	335±110	1200±320	773±120	85	15	7.0±.8
VI. Cholesterol+Nicotine	224±610	296±54	1181±270	747±110	91	9	6.0±.3
VII.Hypert.+Chol.+Nico.	2652±710	420±124	1347±420	885±98	88	12	6.1±.4
Controls	370±82	102±30	71±24	197±66			

LEGENDS

Fig.1A. Coronary angiogram of untreated control revealing filling of right (R) and left coronaries. Filling of the circumflex (C) branch of the latter is greater than that of the descending (D) tributary.

1B. Angiogram from hypertensive, cholesterol-fed rabbit revealing foci of narrowing (arrows) in circumflex branch. The descending and right coronaries do not exhibit as much filling or tortuosity as noted in the control.

Fig. 2A. Selective left coronary angiogram in untreated control.

2B. The angiogram of hypertensive, cholesterol-fed rabbit discloses focal narrowing and irregularity (arrows) of circumflex and descending branches of the left coronary.

Fig. 3. Schematic presentation of atherosclerosis in aorta of (A) normotensive, cholesterol-fed rabbits with and without nicotine administration, (B) hypertensive, cholesterol-fed rabbits with and without nicotine, and (C) non-cholesterol-fed rabbits with hypertension and/or nicotine.

Fig. 4. Cross sections of extramural branches of coronary artery from (A) non-cholesterol-fed, nicotine-treated rabbit. The appearance is similar to that of untouched controls, (B) non-cholesterol-fed, hypertensive rabbit. The luminal area is larger than that noted in A, and (C) hypertensive, cholesterol-fed rabbit revealing intimal cushion and plaque. Orcein elastica X82.

Fig. 5. Appearance of lipid cushions occluding lumens of intramural coronary branches of cholesterol-fed rabbit. H&E X 150.

Fig. 6. Miliary necrosis of myocardium in hypertensive, cholesterol-fed

LEGENDS (contd)

rabbit. H & E X 40.

Fig. 7. Higher magnification of focus of myocardial necrosis depicted in Fig. 7. H & E X 240.